

Protein and DNA Content in the Gills of *Labeo rohita* During Aeromoniasis

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Abstract: *Aeromonas liquefaciens* is one of the most important pathogen of warm water fish. Aeromoniasis is associated with tail and fin rot, hemorrhagic septicemia, exophthalmus, plaes gills etc. It is a serious disease causing heavy damage in Indian Major carp culture. A wide genetic variation in resistance has been noted in *Labeo rohita* for aeromoniasis. The present investigations are designed to estimate the content of protein and DNA from gills of fish experimentally infected with various doses of *A. liquefaciens*. Four groups (66 fish in each group) of fish were given infection intramuscularly @ 10^{-2} CFU/fish (group A), 10^{-4} CFU/fish (group B), 10^{-5} CFU/fish (group C), and 10^{-6} CFU/fish (group D). Another four groups of fish (a, b, c, and d) (66 in each group) were kept as uninfected controls for comparison. Six fish from groups A, B, C and D and a, b, c and d were necropsied, tissues of gill were separated and analyzed for protein and DNA content at hour 1, 3, 6, 12, 18, 24, 36, 48, 72, 96, and 216 and for histopathological changes on hour 24 of experimental period. Marked increase of protein in gill was found at hour 6 and 72 in group A, at hour 1, 18, 24 and 36 in group C and at hour 1 to 216 in group D when compared with controls. A peak level of protein was found at hour 3, 6 and 12 in fish received 10^{-6} CFU/fish (group D) when compared with other experimental groups. The decrease in the content of DNA during later period of infection (from hour 12 to 216) in experimental groups of A and B (which received higher doses) may reflect the depressed oxidative respiration, due to infective bacterial pathogen exposure. Fish received small doses showed much disturbed oxidative respiration under infection gills of experimental fish showed enlargement of lamellae and loss of secondary lamellae (in groups A and B) and atrophy of primary lamellae and curling and clubbing of secondary lamellae (in groups C and D) exposure. Varied degrees of response were observed to various degrees of pathogenic doses in fish with regard to protein and DNA content.

I. INTRODUCTION

Aeromonas infections are regarded as the most common bacterial infections in freshwater fish. There are several species of *Aeromonas* which can infect fish; one among the pathogenic species of *Aeromonas* is *Aeromonas liquefaciens*. *Aeromoniasis* is characterized by hemorrhagic septicemia, abdominal dropsy, fin rot and tail and snout erosions in cultured fish (Kanai and Takagi; 1986; Karunasagar et al., 1986; Lakshmanan et al; 1986). *Aeromonas* are generally found in the gastrointestinal tract of fish and are considered as opportunistic pathogens (Swann and White, 1989; Yildiz et al., 2005). Abnormal conditions of the pond environment like stress, over crowding, temperature fluctuations, poor water quality, high nitrite and carbondioxide levels and mishandling of fish are found to be associated with disease outbreaks (Dixon and Issvoran, 1993; Aoki, 1999; Cipriano, 2001; Lakshmanaperumalswamy et al., 2005; Yildiz et al., 2005). Histopathologically, fish may exhibit haemorrhages on the gill, ulcers in the dermis and tissue damage in liver and kidney. Histopathological studies are recognized as biomarkers in the evaluation of the health of fish exposed to contaminants/pathogenic microbes/metazoan parasites, both in the laboratory (Wester and Canton, 1991; Thophon et al., 2003; Parikh et al., 2010) and field studies (Hinton and Lauren, 1993; Schwaiger et al., 1997; Teh et al., 1997). The histopatological studies on various target organs like gill, kidney and liver are useful to assess the damage to fish and animal health (Hinton and Lauren, 1993; Gernhofer et al., 2001). *Ictalurus punctatus* infected with *A. hydrophila* showed haemorrhagic septicemia and marked histopathological changes in gill – sloughing of respiratory epithelium (Miyazaki, 1985). In the early stages of infection, there were no external skin ulcers in diseased fish. Gill, liver and pancreas showed marked damage in channel cat fish infected with *A. hydrophila* complex (Grizzle and Kiryu, 1993). The Present study is designed to estimate the level of Protein, DNA and histopathological changes in the gills of fresh water fish *Labeo rohita* exposed to different doses of *Aeromonas liquefaciens*.

II. MATERIALS & METHODS

Labeo rohita (approximately 5 months of age; 65-75 gmwt) were procured from K.N. Rao fish breeding centre, Nandivelugu (Guntur District), AP, India and acclimatized in the laboratory conditions and fed with commercial pallets. Virulent strain of *A. liquefaciens* was obtained from MTCC 2654, Chandigarh and pure cultures of bacteria were obtained following streak plate method. Preparation of innulum and infection were made under aseptic conditions.

Four groups (66 fish in each group) of experimental fish were given infection intramuscularly below the dorsal fin of the fish @ 10^{-2} CFU/fish (group A) 10^{-4} CFU/fish (group B), 10^{-5} CFU/fish (group C) and 10^{-6} CFU/fish (group D). Another four groups (a, b, c and d) of fish (66 in each group) were kept as uninfected control for comparison. Six fish from each group were taken and necropsied and total protein and DNA content from gills were estimated following the method of Lowri et al; (1951) and diphenyle amine method. following standard methods. Part of the tissue were fixed in Bouines fluid sectioned (5 μ m) and stained by H and E method for histopathological observations. Results were analyzed using student 't' test.

III. RESULTS AND DISCUSSION

All the experimental fish were observed daily for disease symptoms and found loss of scales, hemorrhages, red mouth, swollen abdomen and excessive secretion of mucus. Fish infected with 10^{-2} and 10^{-3} CFU/fish *A. liquefaciens* showed skin ulcerations and open wound at day 2 and 4 of infection. The results on the content of protein and DNA in gills are shown in table 1.

Protein activity in gill: The content of protein showed marked increase (on hour 3, 6 and 72) and decrease (on hour 1, 12 to 48, 96, 216) in group A. The level of protein was found to decrease from hour 1 to 216 of infection period in group B. The protein value was found to be higher from hour 1 to 48 and lower from hour 72 to 216 when compared with controls in group C. Fish of group D showed higher protein value throughout the experimental period (from hour 1 to 216) in comparison with controls.

DNA activity in gill: The content of DNA was found to be increased at hour 1 and decreased from hour 3 to 216 when compared to normal levels in group A. DNA showed higher values during the initial (from hour 1 to 6) and later (from hour 72 to 216) period of infection in group B; the content of DNA was at normal values from hour 12 to 24 and below normal at hour 36 and 48. It is of interest to note that fish received low doses of infection (group C 10^{-5} CFU/fish; group D, 10^{-6} CFU/fish) showed greater increase of DNA content from hour 1 to 216 in comparison with controls. Statistically analysis (table 2) showed significant increase of protein in groups B, C and D and DNA in groups A and C when compared with controls and among themselves (except the protein value in between A and B).

Histopathological observations in gills: The gill rachis was normal and non-proliferated and numerous semi-circular secondary gill lamellae are lined up along both sides of the gill filament in case of controls. In all the experimental groups of fish suffering due to aeromoniasis, gills showed degenerative changes like vacuolar degeneration, cloudy swelling and necrosis (plate 1). Infected gill showed enlargement of primary lamellae and loss of secondary lamella, in fish received higher doses (in groups A and B). The terminal ends of primary gill lamellae showed marked splitting, and atrophy of primary lamellae and curling and clubbing of secondary lamellae was evident in groups received low doses (groups C and D). Clinical symptoms of aeromoniasis like scale erosion, haemorrhagic skin, distended abdomen and ulceration around mouth, as well as tail and fin rot were observed in all the groups of infected fish. The gross signs of the disease in experimentally infected fish in the present study were similar to those reported earlier (Cipriano *et al.* 1984; Austin and Austin, 1993; Mohanty *et al.*, 2008). Yogananth *et al.*, (2009) reported that pathogenesis caused by *A. hydrophila* in fish can be attributed to several exotoxins secreted by the infectious organisms. The present study explains that *L. rohita* responded to varied doses of *A. liquefaciens* by altering the level of protein and DNA and the histoarchitecture in gill. Marked increased of protein in gill was found on hour 6 (40.0 mg/ml) and 72 (40.34 mg/ml) in group A (10^{-2} CFU/fish); on hour 1 (40.0 mg/ml), 18 (41.03 mg/ml), 24 (40.34 mg/ml) and 36 (46.34 mg/ml) in group C (10^{-5} CFU/fish) and on hour 1 (41.37 mg/ml) to 216 (39.35 mg/ml) in group D (10^{-6} CFU/fish) when compared with controls. The peak level of protein was found on hour 3 (44.48 mg/ml), 6 (44.48 mg/ml) and 12 (44.13 mg/ml) in fish received 10^{-6} CFU/fish (group D) when compared with other experimental groups. The present findings reveal that when there was an increase of protein, the DNA content increased and when there was a decrease of protein, the DNA content decreased in the gill. The stress caused by aeromoniasis might have exerted ill effect in the gill tissue at physiological, cellular and molecular level as evident by the alterations in protein and DNA level (quantitative estimation) and histopathological reactions. These results confirm the observations of Begum (2004) who also observed biochemical alterations in liver and muscle tissue in fish, *Clarius batracus* during insecticide treatment. It was assumed that decrease in protein content might be due to the impaired metabolism as suggested by Miyamoto (1976) and Murty and Devi (1982) in fish exposed to toxicants. Depletion of tissue protein in fish exposed to various toxicants has been reported by several investigators (Durairaj and Selvarajan, 1992; Veeraiah and Durga Prasad, 1998). Decreased protein content suggests the occurrence of intensive proteolytic changes which inturn contributes to the elevation of aminoacids; the reduced level of protein might be due to reduction in protein synthesis as opined by Das and Bhattacharya (2006). The apparent decrease of total protein content under high and low doses of *A. liquefaciens* may be due to detoxification of enzymes. The decreased tendency of total protein may also be due to the metabolic utilization of ketoacids to gluconeogenesis pathway for the synthesis of glucose or may be due to direction of the synthesis of protein from free amino acids as suggested by Neilson (1975). The decrease of protein in the gill is due to the decrease of RNA in the tissues of fish. Holbrook (1980) also reported that the decrease of amino acid incorporation and disaggregation of polysomes lead to the decrease in protein synthesis. These results confirm that of Erer (1981) who reported that carps experimentally infected (intramuscularly) with *A. hydrophila*, infected, showed pathological changes mostly on their skins and muscle. Changes caused in gill by the toxic substances produced by the bacterial

pathogen confirm that of Kanai and Takagi (1986) who observed hemolysis and anasarcous changes in carps which have been intramuscularly infected with *A. hydrophila*

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Table 2: ‘t’ values obtained for different groups of fish infected with 10⁻² (group A), 10⁻⁴ (group B), 10⁻⁵ (group C) and 10⁻⁶ (group D) CFU/fish and Control (groups a,b,c and d)

Experimental (A, B, C and D) and Control (a, b, c and d) groups							
A	a	B	b	C	c	D	d

Gill Protein									
Mean	36.95	38.62	34.81	37.97	39.47	37.97	42.68	37.98	
	A	a	B	b	C	c	D	d	
t vlaue	 t=1.87 [@] (P>0.05)		 t=9.77* (P<0.05)		 t=2.51* (P<0.05)		 t=7.17* (P<0.05)		
	A	B	A	C	A	D			
	 t=2.20 [@] (P>0.05)		 t=2.44* (P<0.05)		 t=5.24* (P<0.05)				
	B	C	B	D	C	D			
	 t=4.82* (P<0.05)		 t=12.83* (P<0.05)		 t=2.82* (P<0.05)				
Gill DNA									
Mean	6.05	8.78	9.92	8.86	15.96	8.77	12.15	8.76	
	A	a	B	b	C	c	D	d	
t value	 t=4.03* (P<0.05)		 t=1.72 [@] (P>0.05)		 t=5.70* (P<0.05)		 t=4.84 [@] (P>0.05)		
	A	B	A	C	A	D			
	 t=4.12* (P<0.05)		 t=6.95* (P<0.05)		 t=6.34* (P<0.05)				
	B	C	B	D	C	D			
	 t=4.26* (P<0.05)		 t=2.70* (P<0.05)		 t=2.45* (P<0.05)				

P value at 5% level of significance is 2.306

*Statistically significant values

[@] Statistically non-significant values

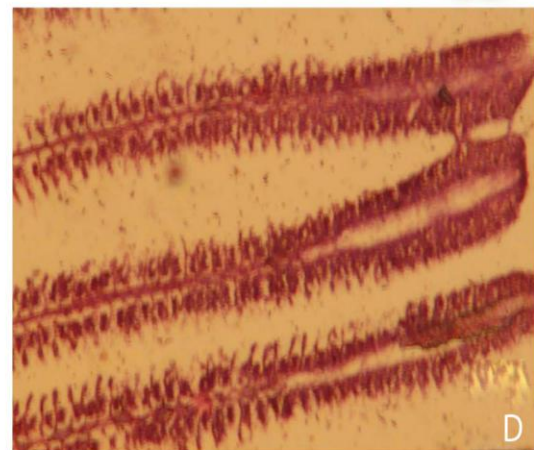
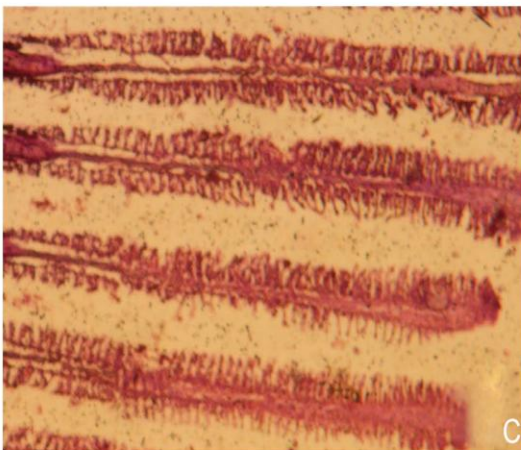
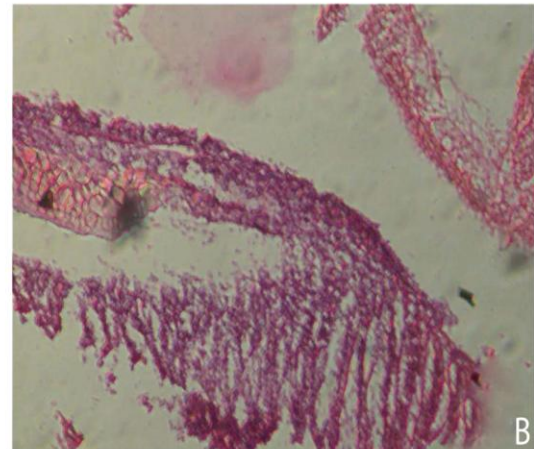
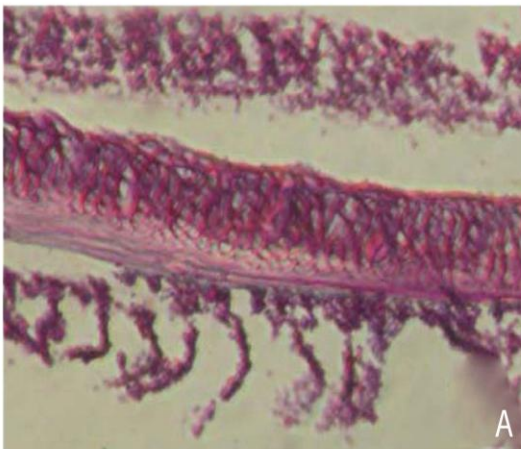
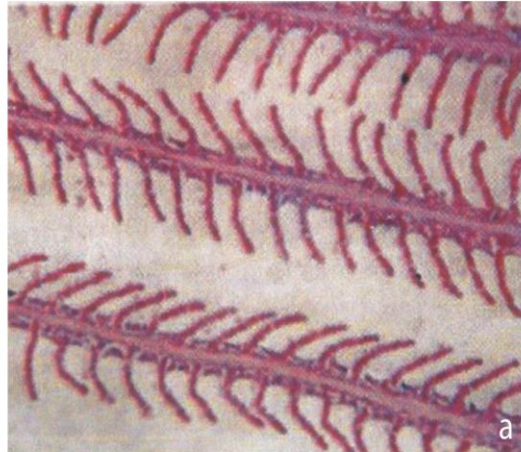


Figure : Showing gill from control (a) and infected (A,B,C and D) fish.

Figure a : The normal structure of gill (10x)

Figure A : Enlargement of primary lamellae and loss of secondary lamellae (20x)

Figure B : Damage of primary and secondary lamellae (20x)

Figure C : Clubbing of primary lamellae and curling of secondary lamellae (20x)

Figure D : Atrophy of primary lamellae and curling and clubbing of secondary lamellae (20x)

Table 2: Protein (mg/ml) and DNA (mg/ml) content in the gill, of experimental fish treated with *Aeromonas liquifaciens* @ 10^{-2} CFU/FISH (group A), 10^{-4} CFU/FISH (group B), 10^{-5} CFU/FISH (group C) and 10^{-6} CFU/FISH (group D) at different periods of infection and control (groups a,b,c and d). Values are expressed in mean derived from five observations.

Hours of Necropsy	Experimental groups								Control groups							
	Group A		Group B		Group C		Group D		Group a		Group b		Group c		Group d	
	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA
1	35.51	10.0	37.58	12.22	40.0	21.11	41.37	12.22	37.99	8.88	37.99	8.88	38.01	8.88	37.98	8.88
3	39.65	5.55	36.89	12.22	39.31	18.88	44.48	13.66	37.97	8.81	37.98	8.81	38.01	8.89	37.97	8.81
6	40.0	4.44	34.82	11.11	39.31	22.22	44.48	14.20	37.96	8.88	37.97	8.88	38.02	8.90	37.96	8.88
12	38.96	5.55	34.82	8.88	39.31	17.77	44.13	16.66	37.98	8.87	37.98	8.88	38.04	7.99	37.98	8.88
18	32.41	2.22	34.48	8.88	41.03	18.88	43.44	16.66	37.97	8.86	37.96	8.82	38.05	8.87	37.98	8.86
24	31.72	7.77	33.79	8.88	40.34	11.11	43.10	15.55	37.98	8.83	37.97	8.81	38.06	8.86	37.99	8.87
36	36.20	7.77	33.44	7.77	46.34	13.33	42.75	10.00	37.99	8.85	37.98	8.83	38.01	8.88	37.98	8.86
48	35.51	5.55	33.79	5.55	39.31	10.0	41.72	10.0	37.98	8.88	37.99	8.82	38.01	8.90	37.98	8.85
72	40.34	6.56	34.13	10.0	37.58	12.22	41.03	10.55	37.99	8.87	37.96	8.88	38.01	7.98	37.98	8.86
96	38.27	7.77	34.37	11.11	37.58	16.66	40.34	10.77	37.99	8.88	37.99	8.87	38.02	7.99	37.99	8.87
216	37.89	3.33	34.82	12.55	34.13	13.33	39.35	10.0	37.97	8.87	37.99	8.88	38.01	7.99	37.98	8.88